

AMENDMENTS TO THE SPECIFICATION

Please replace the title of the application beginning at page 1, line 1, with the following amended title:

**METHODS FOR MODULATING STIMULATING T CELL UNRESPONSIVENESS
RESPONSIVENESS IN TUMOR CELLS.**

Please replace the paragraph, beginning at page 1, lines 8-12 , with the following amended paragraph:

Related Applications

This application is a continuation application of U.S. application No. 08/457,483, filed June 1, 1995, now U.S. Patent No. 6,451,305B1, which is a continuation in-part of U.S. Application Serial No. 08/207,932, filed March 8, 1994, now abandoned entitled "Methods for Modulating T Cell Unresponsiveness" and corresponds to International Application Serial No. PCT/US95/02916, filed March 8, 1995. The contents of these applications is are incorporated in their entirety herein by reference.

Please replace the paragraph, beginning at page 3, lines 11-21 , with the following amended paragraph:

One embodiment of the invention involves maintaining T cell anergy by contacting anergized T cells with an agent which inhibits stimulation of the T cells through CD2. CD2 inhibitory agents include agents which inhibit an interaction between CD2 and a CD2 ligand (e.g., LFA-3, CD48 or CD59). Such agents include blocking antibodies, soluble forms of CD2 and CD2 ligands, peptides and small molecules. Alternatively, a CD2 inhibitory agent can act intracellularly to inhibit an intracellular signal triggered in the T cell through CD2. Another embodiment of the invention involves reversing T cell anergy by contacting anergized T cells with an agent which stimulates the T cells through CD2. CD2 stimulatory agents include a cell which expresses a CD2 ligand on its surface (e.g., LFA-3, CD48 or CD59), ~~multivalent~~

multivalent forms of a CD2 ligand and stimulatory anti-CD2 antibodies. Alternatively, a CD2 stimulatory agent can act intracellularly to trigger a signal through CD2.

Please replace the paragraph, beginning at page 3, lines 22-37, with the following amended paragraph:

The methods of the invention are useful therapeutically in situations where it is desirable to modulate antigen-specific immune responses, e.g., maintain antigen-specific T cell unresponsiveness or restore antigen-specific T cell responsiveness. For example, it may be necessary to maintain T cell unresponsiveness in a subject who has received an organ or bone marrow transplant to prevent graft rejection by inhibiting stimulation through CD2. In addition, T cell unresponsiveness can be maintained by blocking CD2 stimulation in a subject who has an autoimmune disease to alleviate symptoms of the autoimmune disease. In these cases, a CD2 inhibitory agent is administered to the subject in an amount and over a period of time sufficient to maintain T cell unresponsiveness. Alternatively, T cell unresponsiveness can be reversed in a subject bearing a tumor to stimulate a tumor-specific T cell response or in a subject receiving a vaccine to enhance the efficacy of the vaccine. For example, a cell (e.g., a tumor cell) can be modified to express a CD2 ligand or a CD2 stimulatory agent can be administered to the subject bearing a tumor or who has had a tumor surgically removed to prevent recurrence of ~~th~~ the tumor. Additionally, antigen-specific responsiveness can be restored to anergized T cells *in vitro* by stimulating the T cells through CD2. Responsive T cells generated *in vitro* can then be administered to a subject.

Please replace the paragraph, beginning at page 5, lines 18-27, with the following amended paragraph:

One aspect of this invention pertains to methods for maintaining T cell unresponsiveness which are particularly useful in therapeutic situations in which T cell anergy has been induced and it is desirable to preserve the antigen-unresponsive state. Examples of such therapeutic situations include recipients of allogeneic or xenogeneic cell or tissue, such as an organ and bone

marrow transplant and subjects having an autoimmune ~~diseases~~ disease. In these situations, a therapeutic regimen may have induced a state of antigen-specific T cell unresponsiveness in the subject to be treated. For example, antigen-specific T cell unresponsiveness can be induced in a subject by inhibiting a costimulatory signal in T cells, e.g. with an agent CTLA4Ig (see e.g. Turka, L.A. et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:11102-11105; Lenschow, D.J. et al. (1992) *Science* 257:789-792).

Please replace the paragraph, beginning at page 8, line 35, through page 9, lines 1-5, with the following amended paragraph:

In one embodiment, a soluble form of CD2 or a CD2 ligand is a ~~truncated~~ truncated form of the molecule comprising an extracellular domain of the molecule or a functional portion thereof. A portion of the extracellular domain of CD2 which retains the ability to bind to a CD2 ligand can be used. Likewise, a portion of the extracellular domain of a CD2 ligand which retains the ability to bind to CD2 can be used. A soluble truncated form of CD2 or a CD2 ligand can be obtained using standard recombinant DNA techniques, for example by introducing into host cells a recombinant expression vector including a nucleotide sequence encoding the extracellular domain of the molecule, or a functional portion thereof, in a form suitable for expression and secretion of the extracellular domain by the host cells, and isolating the extracellular domain (or portion thereof) from the culture medium.

Please replace the paragraph, beginning at page 9, lines 6-25, with the following amended paragraph:

A recombinant truncated form of CD2 or a CD2 ligand can be designed based upon the nucleotide sequence encoding the protein, by standard techniques. The nucleotide sequence encoding human CD2 is disclosed in Seed, B. and Aruffo, A. (1987) *Proc. Natl. Acad. Sci. USA* 84:3365-3369. Two forms of the LFA-3 molecule have been described including a typical transmembrane protein. A nucleotide sequence encoding the trans-membrane form of LFA-3 is described in Wallner, B.P. et al. (1987) *J. Exp. Med.* 166:923-932. The second form is a

glycosyl phosphatidylinositol (GPI)-linked protein. A nucleotide sequence encoding this form of LFA-3 is disclosed in Seed, B. (1987) *Nature* 329:840-842. The GPI-linked form of LFA-3 is anchored to cell membranes by a phospholipid tail. Thus, a soluble form of LFA-3 can be obtained, for example, either by recombinant expression of the extracellular domain of the protein or by cleavage of the phospholipid tail of the GPI-linked form (e.g., using phosphatidylinositol-specific phospholipase C) from the surface of cells expressing this form of LFA-3 to release a soluble form of LFA. Another CD2 ligand, CD48, is a typical transmembrane protein. A nucleotide sequence encoding CD48 is described in Korinek, V. et al. (1991) *Immunogenetics* 33:108-112. Similar to the GPI-linked form of LFA-3, CD59, another CD2 ligand, is a phosphatidylinositol-anchored membrane protein. CD59 can ~~therefor~~ therefore be cleaved from the surface of cells expressing the ligand by phospholipase treatment to obtain a soluble form of CD59. Alternatively, the extracellular domain of CD59 can be expressed recombinantly. A nucleotide sequence encoding human CD59 is disclosed in Sawada, R. et al. (1990) *DNA Cell. Biol.* 9:213-220.

Please replace the paragraph, beginning at page 12, lines 1-15, with the following amended paragraph:

A CD2 inhibitory agent is administered to subjects in a biologically compatible form suitable for pharmaceutical administration *in vivo*, in an amount and for a time sufficient to maintain T cell unresponsiveness in the subject. By "biologically compatible form suitable for administration *in vivo*" is meant a form of the CD2 inhibitory agent to be administered in which toxic effects are outweighed by the therapeutic effects of the agent. Administration of a CD2 inhibitory agent as described herein is in a pharmacological form including a therapeutically active amount of agent alone or in a pharmaceutically acceptable carrier. Administration of a therapeutically active amount of the CD2 inhibitory agent is defined as an amount effective, at dosages and for periods of time ~~necessay~~ necessary to achieve the desired result (e.g., maintain T cell unresponsiveness). For example, a therapeutically active amount of a CD2 inhibitory agent may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of agent to elicit a desired response in the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, several divided doses may

be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

Please replace the paragraph, beginning at page 15, lines 30-37, with the following amended paragraph:

This method is also useful therapeutically, in situations where it is desirable to inhibit an antigen-specific immune response, for example in organ transplantation, bone marrow transplantation and autoimmune diseases. For therapeutic purposes, a CD28/CTLA4 inhibitory ~~agent~~ agent and a CD2 inhibitory agent are administered to a subject to induce and maintain T cell unresponsiveness. These agents can be administered to a subject ~~simultaneously~~ simultaneously or sequentially. Routes of administration, pharmaceutical compositions for administration, timing and dosages of administration and other considerations for administration are as described above for CD2 inhibitory agents.

Please replace the paragraph, beginning at page 16, lines 8-20, with the following amended paragraph:

In one embodiment, T cell responsiveness to an antigen is restored by contacting an anergized T cell in the presence of the antigen with an agent which stimulates the T cell through a CD2 surface receptor. The method provides a means by which T cell responsiveness is fully, or partially restored (as compared to the responsiveness of the T cell prior to being anergized) such that the T cell responds (i.e., proliferates, secretes Il-2) to subsequent stimulation by the antigen and a costimulatory molecule. An agent which stimulates the T cell through a CD2 surface receptor is ~~referred~~ referred to herein as a CD2 stimulatory agent. To restore T cell responsiveness, a CD2 stimulatory agent can be a cell which expresses a CD2 ligand on its surface. Alternatively, a CD2 stimulatory agent can be a soluble, stimulatory form of a CD2 ligand (e.g., a multivalent form or a micellar form). In addition, the CD2 stimulatory agent can be at least one anti-CD2 antibody or a combination thereof. A CD2 stimulatory agent useful in the methods of this invention can also be an agent which acts intracellularly to stimulate an intracellular signal triggered by CD2.

Please replace the paragraph, beginning at page 23, lines 7-15, with the following amended paragraph:

To restore T cell responsiveness to an antigen, it may be necessary to contact T cells with a third agent which primes the T cells for stimulation through CD2. Agents for priming a T cell are described above. A preferred agent for priming T cells and for stimulating exposure of a T11.3 neo-epitope on CD2 on T cells is IL-2. The T cells can be contacted with the agent which primes the T cells for stimulation through CD2 prior to being contacted with ~~the~~ an agent which stimulates the T cell through CD2. A T cell can be contacted with an agent which primes the T cell for stimulation through CD2 either *in vitro* or *in vivo*. For example, T cells can be obtained from a subject and cultured *in vitro* in IL-2 prior to being readministered to the subject or IL-2 can be administered systemically to a subject.

Please replace the paragraph, beginning at page 23, lines 16-34, with the following amended paragraph:

In a preferred embodiment, the invention provides a method for restoring a tumor-specific T cell response in a tumor-bearing subject comprising modifying tumor cells to express a CD2 ligand and a CD28 or CTLA4 ligand. Preferably, tumor cells are modified to express a CD2 ligand and a CD28 or CTLA4 ligand by introducing into the tumor cells at least one nucleic acid encoding the CD2 ligand and the CD28 or CTLA4 ligand in a form suitable for expression of the CD2 ligand and the CD28 or CTLA4 ligand on the tumor cells' surface. Tumor cells can be modified *in vivo* or *ex vivo*. For example, a tumor cell can be modified to express a CD2 ligand *in vivo* by infection of tumor cells with a recombinant virus which encodes the CD2 ligand in a form suitable for expression of the CD2 ligand on the surface of the tumor cell. Alternatively, tumor cells can be removed from a subject, modified *ex vivo* to express a CD2 ligand and readministered to the subject. When modified *ex vivo*, a sample of tumor cells can be modified to express both ligands (i.e., a CD2 ligand and a CD28 or CTLA4 ligand), or alternatively, one sample of tumor cells can be modified to express a CD2 ligand and a second sample of tumor cells can be modified to express a CD28 or CTLA4 ligand. The two samples of tumor cells can then be administered to a subject simultaneously or sequentially. It may be beneficial to first administer a sample of tumor cells which express a CD2 ligand to restore

tumor-specific responsiveness, followed by administration of a sample of tumor cells which express a CD28 or CTLA4 ligand to stimulate ~~an~~ a tumor-specific T cell response.

Please replace the paragraph, beginning at page 24, lines 3-14, with the following amended paragraph:

Accordingly, this invention also provides tumor cells modified to express a CD2 ligand, wherein the tumor cells do not express the CD2 ligand prior to modification. Preferably, the tumor cells are modified to express LFA-3. The invention also provides a tumor cell which is modified to express a CD2 ligand and a CD28 or CTLA4 ligand, wherein the tumor cell does not express the CD2 ligand and the CD28 or CTLA4 ligand prior to modification. Tumor cells modified to express LFA-3 and B7-1 or B7-2 are preferred. Tumor cells can be modified to express a CD2 ligand and optionally a CD28 or CTLA4 ligand as previously described. Modified tumor cells can be incorporated into a composition suitable for pharmaceutical administration. For example, tumor cells can be administered in a pharmaceutically acceptable carrier or diluent (e.g., a sterile saline solution). Tumor cells can be administered by an ~~appropriate~~ appropriate route which delivers the tumor cells to a desired location, e.g., by intravenous, subcutaneous, intramuscular or intraperitoneal injection.

Please replace the paragraph, beginning at page 26, lines 22-23, with the following amended paragraph:

EXAMPLE 3: ~~**Maintenance**~~ **Maintenance** of T Cell Unresponsiveness by
 Blockage of a CD2/LFA-3 Interaction